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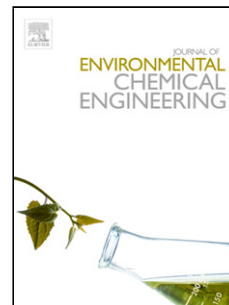
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Low-cost post-treatments improve the efficacy of hydrochar as peat replacement in growing media

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Abstract

Peatlands represent a valuable global carbon store and are critical for preserving biodiversity. In order to reduce peat mining a significant effort is made to search for substitutes of peat in its different uses. Organic products obtained by hydrothermal carbonization processes could fully or partially replace peat in growing media if they possess some suitable properties. The study focused on the properties of a hydrochar produced from wheat (HTC) and included its chemical characterization and stability estimates using microbial respiration and nitrogen mineralization essays. As inhibition to seed germination could significantly restrict the eventual use of HTC in growing media, a number of seed germination trials were carried out to reveal the magnitude of phytotoxicity in relation to sphagnum peat and biochar and the effect that some simple and low-cost pre-conditioning treatments have on the rate of germination. This rate was greatly increased by simply wetting the material a few days prior to use or by mixing it with compost. It was shown that the positive effect on seedling emergence should be attributed, at least partly, to the degradation by microorganisms of toxic substances presumably produced during carbonization.

Keywords: hydrothermal carbonization; peat replacement; growing media; seed germination trial; stability

1. Introduction

It is widely recognized that peat bogs are valuable habitats that need to be preserved for wildlife and because their drainage and peat exploitation represent a significant source of carbon dioxide emissions. One of the main uses of peat harvested from these bogs is in the growing media industry where peat currently represents about 77-80% of the materials used annually in Europe [1]. Significant efforts are made, therefore, for peat replacement by alternative suitable materials.

In order for other organic materials to be used as growing media and fully or partly replace peat they need to fulfill certain requirements concerning their physical, chemical and biological parameters such as Electrical Conductivity (EC), pH, stability and nutrient release. Important functions that all growing media should include are water retention, good aeration, infiltration and nutrient holding capacity. They should not rapidly decompose, have low bulk density and easy flowing to facilitate pot filling. Compost, for example, although possesses most of the above properties, in most cases cannot be used on its own as a growing medium due to its high level of soluble salts and its high pH [2] and is mainly found as a dilution material of peat. Evidently, one of the most important properties of a growing medium is the lack of any phytotoxic effects [3], which are defined as a delay of seed germination, inhibition of plant growth or any adverse effect on plants caused by specific substances (phytotoxins) or growing conditions [4].

Hydrochar could eventually represent a peat replacement material. It is produced by agricultural or municipal waste and sludge in a carbonization process conducted in a closed system under wet conditions at temperatures ranging between 180-250°C and high pressures. It contains around 50-60% of the carbon originally

present in the biomass and under particular conditions of chemical activation a highly porous material can be created [5].

It is already known, though, that during carbonization a number of phytotoxic substances are formed, some of which have been shown to inhibit or reduce germination rate of crops [6, 7]. In hydrochars, the content of PAHs is lower than in biochars, but the dissolved organic carbon (DOC) and the phenolic compounds contents are higher. The three substances identified to severely inhibit germination (glycolic acid, levulinic acid and guaiacol) are generally only found in considerable amounts after hydrothermal carbonization [8].

The need of detoxification before the eventual use of hydrochar as soil amendment or in growing media has generally been acknowledged and a number of after production treatments have already been proposed. They can be grouped in physicochemical and biological treatments. The physicochemical include drying or heating to release toxic volatile substances, washing with hot or cold water and treatment with hydrogen peroxide (H_2O_2) to split toxic compounds into smaller non-hazardous molecules. Kern et al. [9] pointed to the “aging” effect of storage in order to explain the destruction of toxic substances and lessening of phytotoxicity during seed germination of a hydrochar material that received no other treatment. The biological treatments, such as composting or co-composting of hydrochar [10], aim in enhancing the microbial activity on the material increasing also the rate of decomposition of phytotoxic ingredients. All above practices, however, lead inevitably to increased production costs or time of preparation before use.

The objective of this study was to examine the suitability of wheat hydrochar to be used in growing media. It focused on the main biological properties of the material, such as stability and seed germination performance, and analyzed some

simple and low-cost ways with which the latter could be improved. The work aimed also to show that the destruction of toxic substances receiving these treatments, namely wetting of the material a few days before use or mixing with small quantities of compost, was to a great extent microbiological.

2. Materials and Methods

2.1 Production and chemical characterization of materials

The hydrochar material used (referred from now on simply as HTC) was produced in an 18.6 L Parr stirred pressure reactor (model 4555) at the Leibniz Institute for Agricultural Engineering in Potsdam using wheat straw as feedstock. The wheat straw was obtained in bales of dedusted straw with an average cutting length of 35 mm. For hydrothermal carbonization, 600 g of wheat straw and ten liters of de-ionized water were transferred into the reactor. The reactor was operated at the set temperature of 200°C for 180 min and a heating rate of 90 K min⁻¹. The reactor was then left cooling over night to a final temperature of 27-30°C. The resulting hydrochar sludge was sieved and dried and stored in gas-tight state until further use. Feedstock was processed in two separate carbonization runs yielding an average amount of dry HTC of 329.9±0.1 g.

The biochar from maize silage was obtained from Regenerative Energie Wirtschaftssysteme GmbH (Quakenbrück, Germany). It was produced by a continuous Regenis MAX pyrolyzer with a nominal throughput of 150 kg h⁻¹. The pyrolyzer was a staged system with consecutive steps for drying, degassing, and pyrolysis along the horizontal material flow. The biochar used in this study was

produced at a pyrolysis temperature of 600 °C (30 min), a throughput of 100 kg h⁻¹, and mild negative pressure of 5 mbar. Afterwards the hot char was quenched by means of water sprinkling. The biochar produced was stored for several months before use in FIB container outside under a roof.

The compost material used in germination trials was mature compost coming from *Ficus microcarpa* and olive tree prunings produced at the premises of the Agricultural Research Institute of Cyprus. It had an ash content of 54.3%, pH 8.53 and EC 2.83 mS/cm. The fertilized peat that was used for comparison was a medium decomposed sphagnum peat (H5 on von Post scale) with bulk density of 0.095 g cm⁻³.

Above materials were analyzed for pH, EC and extractable (CAT) macronutrients [11, 12, 13]. Total nutrients were determined after drying the samples at 105°C [14].

2.2 Stability measurements

To qualify stability, which is one of the most important biological properties of a growing medium, Oxygen Uptake Rate (OUR) tests and microbial respiration and N mineralization measurements after mixing with soil were used. Results of the first approach rest on the activity of the innate microorganisms found inside the material, whereas the other two on the microbial community brought by soil.

For the OUR estimates the OxiTop® measuring system (WTW, Wilhelm, Germany) was employed. The sample, diluted in water, was put in a glass vessel and due to aerobic microbial activity the oxygen consumption was measured by the pressure heads that are mounted on the vessels. The method followed was the [15]. It is underlined that in order to use 2 g of organic material, the ash and moisture content

of each sample was estimated before each trial. Ash or, by difference, organic matter content, was measured through the loss on ignition (LOI) at 450°C in a muffle furnace for 12 hours.

2.3 Nitrogen mineralization/immobilization

Mixtures of HTC with soil were used to estimate its N mineralization potential in controlled moisture and temperature conditions. Soil used was sampled from a wheat field, air dried and sieved with a 2mm sieve. Visible plant residues and roots were removed by hand. This soil had 0.8% organic C content, 0.085% total N, 15% CaCO₃, 39% sand and 35% clay.

Incubations of soil + HTC mixtures were carried out in small plastic 50 ml containers filled either with 20 g of soil only or with 20 g of soil and 0.5 g of HTC material. In order to reduce evaporation water losses, lots of 5-6 containers were enclosed in glass jars with air tight fittings (the same as those described below for microbial respiration measurements), which were often being opened to replenish oxygen. Water added in containers corresponded to 70% of the WHC of the soil, whereas throughout the incubation temperature was kept at $25 \pm 2^\circ\text{C}$. The amount of mineral N in the soil samples was determined at regular intervals up to 49 days from the onset of incubation. Each time, three samples per treatment, were removed from the incubation chamber.

Soil inorganic N (NH₄⁺-N and NO₃⁻-N) was extracted at each time point with 2N KCl. The whole content of the plastic containers used for incubations was used for extraction. Samples were shaken for 30 min, sieved through No. 2 Whatman filter paper and stored frozen. Inorganic N was determined in extracts by colorimetric

methods. In brief, nitrate determination involved reduction of nitrate to nitrite by a copper-cadmium column. The nitrite was then measured following reaction with a diazotizing reagent (sulfanilamide) and a coupling reagent (N-(1-naphthyl)-ethylenediamine dihydrochloride). The purple colour developed was measured at 550 nm. The estimation of $\text{NH}_4^+\text{-N}$ was based on the emerald green color formed when ammonia and sodium salicylate react in the presence of sodium hypochlorite at high pH. The color reaction was catalyzed by the presence of sodium nitroprusside.

2.4 Microbial respiration

Microbial respiration measurements, acquired at optimum temperature and moisture conditions in the laboratory, were carried out to indicate the susceptibility to microbial attack of the HTC materials.

Soil samples of 70g contained in plastic pots were remoistened by adding water corresponding to 70% of the WHC of soil. Incubations were carried out in 2-L gas-tight jars at 25°C. To maintain a vapor saturated atmosphere inside the jars water was always kept at their bottom. Evolved CO_2 was captured in a vial containing 40 mL of 0.5 mol/L NaOH and the quantity of $\text{CO}_2\text{-C}$ absorbed in the alkali was determined by titration with 0.2 mol/L HCl.

Two incubations were performed. In the first, the respiration of “HTC only”, “HTC + soil” and “soil only” were compared. Plastic pots at the “HTC only” treatments contained 5 g of material, whereas the HTC that was mixed in soil at the “HTC + soil” treatment was 0.5 g. In the second, estimates of CO_2 release were carried out on the same materials that were utilized at the second seed germination

trial described below, to reveal the effect on decomposition rate of the pre-conditioning of HTC with water or compost addition.

2.5 Germination trials

Three germination trials were carried out successively and observational evidence of the initial ones was taken into account in the design of the following. Cress seeds (*Lepidium sativum*) were germinated in plastic trays with 10 x 8 individual plastic pots in each (35 cm³ volume) at a rate of 1 seed per pot which was placed at about 0.5 cm below surface.

The germination index used was a modification of the Munoo – Liisa vitality index – MLV [16, 17]. This index compares the product of germination of seeds in the tested material (%) and the average aboveground seedling biomass in the test and control samples and is calculated according to

$$MLV(\%) = \frac{Gs RLs}{Gc RLc} * 100 \quad [1]$$

where,

MLV is the modified Munoo – Liisa vitality index of the sample (% to control); Gs is the germination rate (%) of the test material after 24 hours; Gc is the average germination rate of the control material (%) after 24 hours; RLs is the average aboveground plant biomass, and RLc is the average aboveground plant biomass of the control material.

2.5.1 First germination trial

The treatments of the first germination trial were: 1) Biochar + 25% compost (v/v), 2) Biochar + 10% compost (v/v), 3) Biochar only, 4) HTC + 10% compost (v/v), 5) HTC only and 6) sphagnum peat. Some of the characteristics of the compost used have been presented above.

2.5.2 Second germination trial

To test the hypothesis that the growth of microorganisms feeding on HTC material would progressively reduce phytotoxicity increasing seed germination rates, the material was watered for a few days or watered and mixed with compost, a material that is known to enhance microbial communities and activities. We use the term pre-conditioning for both these treatments of HTC before seeding.

The treatments of the second germination trial were:

- 1) Pre-conditioning by adding compost (20% v/v) to the HTC and by wetting the mixture 20 days before seeding
- 2) Pre-conditioning of HTC material by wetting it 20 days before the start of the test without compost
- 3) Pre-conditioning by adding compost (20% v/v) to the HTC and by wetting the mixture 10 days before seeding
- 4) Pre-conditioning of HTC material by wetting it 10 days before the start of the test without compost
- 5) Compost addition (20% v/v) to the HTC and wetting just before seeding
- 6) No pre-conditioning.

2.5.3 Third germination trial

There are two plausible explanations of the effect of pre-conditioning on seed germination. The reduction of phytotoxicity attained during this phase could be the result of either microbiological degradation or physico-chemical reduction of concentration, e.g. through volatilization, of toxic substances produced during charring. To examine whether the effect of pre-conditioning is, to a certain extent, a biological effect, the HTC material was kept in chloroform fumes in a closed chamber (fumigation) to not allow the growth of microorganisms and seed germination rates was comparatively assessed in fumigated and non-fumigated samples.

Twelve open glass petri dishes were filled with HTC material and arranged in a vacuum desiccator lined with wet paper towels. At the bottom of the desiccator was put a flask containing 25 mL ethanol-free chloroform and a few boiling chips. The desiccator was connected to a vacuum pump and evacuated until the chloroform boiled for 1 min. The desiccator valves were then closed and the material was incubated in chloroform vapor inside the desiccator at room temperature. After fumigation the desiccators were opened and residual chloroform was evacuated by venting to atmosphere 6 times. The procedure was carried out in a fume hood. The HTC material was either fumigated for 24 h before it was used in germination trials or 10 days. In the last case, the procedure was repeated with new chloroform after 5 days.

The treatments of the third germination trial were: 1) fumigated HTC – time 0, 2) non-fumigated HTC – time 0, 3) Fumigated HTC – 10 days, and 4) non-fumigated HTC – 10 days.

2.6 Statistical analysis

Seed germination data were used to create germination curves to be treated statistically as survival curves (PRISM, Graphpad Software, Inc., San Diego, CA), i.e. the percentage of non-germinated seeds was plotted against time. The statistical tests applied were Logrank and Gehan-Breslow-Wilcoxon tests and the null hypothesis tested was that treatments did not change germination rate. For each germination trial, treatments were compared two at a time.

A nonlinear regression analysis was performed on the cumulative CO₂ respiration curves (data in Figure 3) using the curve-fitting program in GraphPad PRISM (Graphpad Software, Inc., San Diego, CA). The nonlinear functions that were fitted were chosen to represent a simple function (one phase exponential association) and to have the smallest residual sum of squares. Statistical differences between treatments were determined at the 0.05 level ($P < 0.05$) and they were carried out pairwise testing the null hypothesis that one single curve adequately fits both data sets.

For comparisons of cumulative amounts of CO₂ or mineral-N released during the other laboratory incubation experiments (data in Figures 1 and 2a) a *t*-test was performed.

3. Results and Discussion

3.1 Peat replacement in relation to chemical characterization and stability

The material produced from hydrothermal carbonization of wheat straw, a material similar in texture, colour and flowing properties with pipe tobacco, appears to possess the characteristics that are needed in potting materials.

3.1.1 Chemical properties

Chemical characterization results (Table 1) showed that feedstock was a very important factor in determining the chemical properties of materials. Analysis of chemical characteristics showed that HTC tested showed smaller values of pH, EC and $\text{NH}_4\text{-N}$ than the biochar. On the basis of their low pH and low EC, hydrochar materials and biochar appear to be suitable for partial or full peat substitution. It is underlined that the germination of most plant seeds is sensitive to high EC of the growing medium and this is the reason why in germination tests, when negative effects of phytotoxins have to be discerned from the negative effects of EC, the organic substrate under examination is usually diluted with another low EC material like sphagnum peat. On the contrary, the biochar had smaller concentrations of P, Mg, SO_4^- and Na^+ than the hydrochars. The reasonable levels of nutrients could imply some savings on fertilizer expenses relative to peat which has very low levels of plant nutrients.

3.1.2 Stability

OUR estimates using the OxiTop® system showed that the stability of all three materials tested was high and that HTC was characterized by greater resistance to degradation than peat moss but smaller than maize biochar. Peat moss had an O_2

consumption of 7.7, HTC 5.7 and biochar 3.4 mmol O₂ kg⁻¹ O.M. hour⁻¹. Results indicate also that the materials are either inert or “native” microbial communities inside or on the surface of these materials were very small.

As it is generally desired that a substrate in soilless production systems remains stable over lengths of time that range up to several years so as to not modify the root environment of a plant significantly [18], hydrochar and biochar materials can be considered as suitable for growing media.

3.1.3 Nitrogen mineralization

The 50-day incubation of HTC + soil mixture samples showed a clear initial net N immobilization phase that lasted approximately 35 days (Figure 1). This supported previously presented stability estimates indicating that the material is partly decomposable, but due to its high C:N ratio (106:1) microorganisms immobilize soil mineral N. Similar results were found by Bargmann et al. [19], who recommended that the use of hydrochars as amendment in arable field or horticultural pot production will require an adjustment of N-mineral-fertilization strategies. In field and pot trials, [20] also showed that the incorporation of hydrochars in soil reduced initial sugar beet growth, especially when this hydrochar had a high C:N ratio and that fertilizer N supply partly compensated for the reduced seedling growth. The authors suggested N immobilization as the most plausible explanation of reduced plant growth. Grunert et al. [21] showed that an organic growing medium can be improved in terms of its nitrogen dynamics by selecting and mixing the appropriate growing media components, which ultimately determine microbial nitrogen conversions.

3.1.4 Microbial respiration

Figure 2a show estimates of CO₂ release from decomposition of soil, pure HTC and mixtures of HTC and soil in incubation trials. Results show that at least a certain fraction of HTC is decomposable. When respiration from “soil only” was subtracted from the mixture of soil with HTC it was possible to obtain CO₂ release rate aided by the activity of soil microbial community and to compare this rate with the one achieved from “native” microorganisms. It was clear that mixing HTC in soil boosted its decomposition rate significantly ($P < 0.05$, Figure 2b). Increased CO₂ emissions after incorporation in soil were also found by [22], which were attributed to the labile fraction of hydrochar. Similarly, Bamminger et al. [23], showed a certain degree of decomposability of hydrochar since its additions in arable soil induced positive priming effects, stimulating the degradation of soil organic carbon (see also review by [9]).

3.2 HTC as a substrate for seed germination

The results from the three seed germination trials that are presented below clearly indicate a certain degree of phytotoxicity of HTC presumably due to substances produced during carbonization. However, some simple and low-cost pre-conditioning treatments may improve the rate of seed germination. The positive effect of these treatments should be attributed, at least partly, to the microbial degradation of toxic substances.

3.2.1 First germination trial

First seed germination trial results (Table 2) showed that HTC is a better medium for seed germination than biochar when materials have not received any treatment. Mixing of HTC or biochar with compost improved germination rate (and initial growth of plants). Such an effect was obtained for HTC with only 10% (v/v) addition of compost, whereas for biochar a greater proportion of compost (25% v/v) was needed. The mixture of HTC with compost was shown to be the best medium for plant growth including that of the mixture of biochar with compost (25% v/v). Apparently, the addition of compost aided the degradation or simply the dilution of toxins that were present inside the organic materials.

3.2.2 Second germination trial

It is already well documented that significant plant growth stimulating effects are obtained after storage or washing of hydrochar. Although mechanisms that are responsible for the positive effects of these post-treatment need further elaboration it is clear that after these treatments smaller phytotoxic effects are observed [8, 24].

This second germination trial aimed in providing greater insight into the process of improvement in quality of HTC as growing substrate after pre-treatment. Results showed that either compost addition or wetting 10 or 20 days prior to seeding improved germination rate and initial growth of plants (Table 3). However, the addition of compost did not add an extra benefit to germination rate in relation to material that was pre-conditioned for 10 or 20 days with water. It is obvious, therefore, that the improvement in germination rates cannot be the result of simply the dilution of toxins, since equally positive results were obtained by only wetting the

material and allowing the growth of microbial biomass. The growth of saprophytic fungi was often observed on HTC or HTC + compost mixtures when they were stored humid (see also [3]). Results showed also that there was no effect of the number of days of pre-conditioning.

Figure 3 shows the results of cumulative microbial respiration of HTC materials alone or after mixing them with compost at time 0 or after a 10-day or 20-day pre-conditioning. Similarly with seed germination results, greatest respiration rate in both “HTC only” and “HTC + compost” treatments was not shown by the 20-day but the 10-day pre-conditioned materials. Presumably the thorough colonization of material by microorganisms and destruction of substances such as sugars, organic acids but also soluble toxic compounds impeding decomposition resulted in greater CO₂ release at the 10-day treatment in relation to 0-day treatment while the exhaustion of the easily decomposable organic fraction of this material reduced respiration after 10 more days of pre-conditioning. The addition of compost increased microbial respiration at all pre-condition times.

Continued decomposition when organic materials such as hydrochars are added to soil or growth media may have negative impacts on plant growth reducing oxygen or nitrogen availability. Microbial decomposition may also pose problems during storage as anaerobic microsites can lead to odors or the production of toxic compounds. The rapid exhaustion of the labile material found in this study indicated that such risk is limited, restricting thus also eventual trading problems arising from the storage of a product, particularly in bags, that is constantly decreasing in mass and volume.

3.2.3 Third germination trial

Fumigation by itself did not seem to affect the germination capacity of seeds since twenty four hour fumigated samples showed similar germination rates in relation to non-fumigated material. On the contrary, there was a significantly higher germination rate in the material that was simply stored moist for 10 days without fumigation in relation to the material that was kept moist for 10 days but without allowing the development of microbial activity (10-day fumigated samples). At the end of the 10-day period non-fumigated HTC had been covered by fungi hyphae. These results (Table 4) clearly indicate that the process of phytotoxicity reduction after pre-conditioning with water is at least partly biological.

All three trials combined lead to the conclusion that there are two supplemental variables controlling seed germinations rates, but acting with varying intensity: i) the concentration of toxins, which have been produced during charring and which are progressively degraded by microorganisms, and ii) the magnitude of microbial activity of the growing medium.

4. Conclusions

Wetting HTC a few days before use or mixing it with compost seem two very promising practices as they help the destruction of phytotoxic compounds of HTC increasing germination rates. They provide two more practical, quicker and less costly ways of material pre-conditioning than co-composting, washing or heating. Although further research is needed on the long-term behavior of the mixture, the addition of compost does not lead to a significant reduction of the lifetime of the HTC in which it

is added because its positive impact on decomposition is halted once the labile fraction of the material is exhausted.

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Figure captions

Fig. 1: Inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) extracted from soil alone or soil + HTC mixture in relation to time of incubation. Each point is the mean of three replicates and bars associated with the points represent the standard error of the mean.

Fig. 2: **a)** mg of C-CO₂ per g of dry material released when “HTC only”, “soil only” or the mixture of them were incubated at optimum temperature and moisture conditions. **b)** CO₂ release (mg of C-CO₂ per g of dry material) coming from HTC incubated alone in comparison with respiration from HTC obtained when CO₂ from “soil only” of Fig. 2a was subtracted from the mixture of soil + HTC.

Fig. 3: Cumulative C-CO₂ release (mg C-CO₂ g⁻¹ dry material) during incubation under optimum conditions of the same materials that were used at the second seed germination trial resulting from different combinations of days of wetting prior to seeding and compost addition (20% v/v). For detailed description of treatments see text. Each point is the mean of three replicates and bars associated with the points represent the standard error of the mean. Curves followed by the same letter are not significantly different between them.

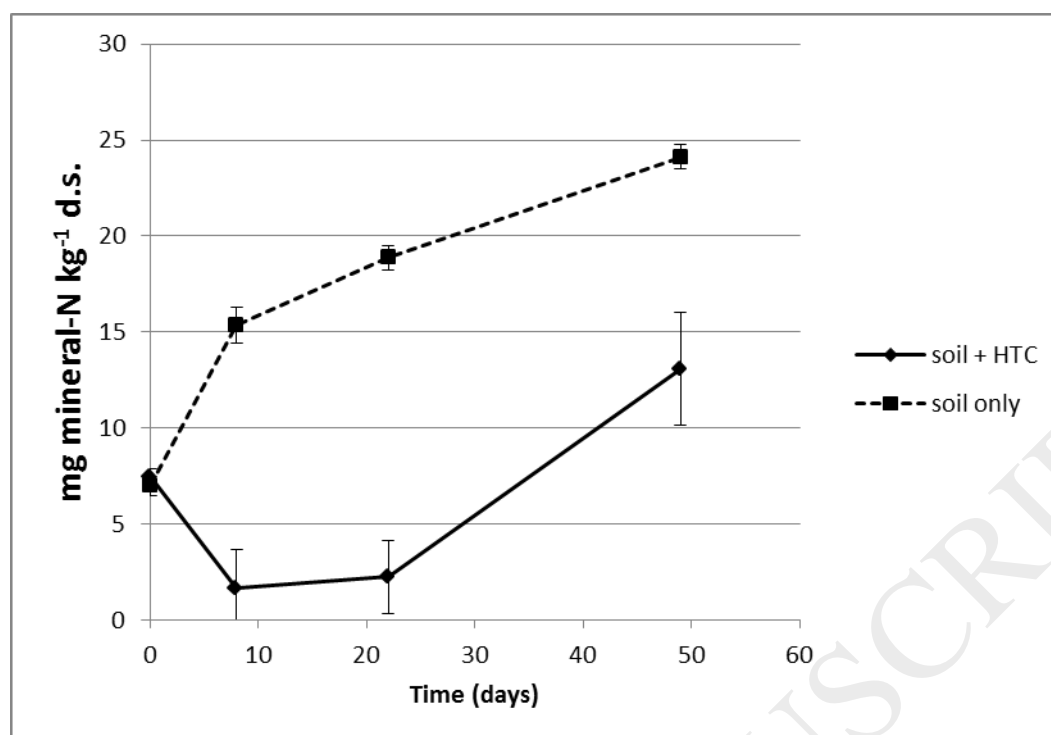
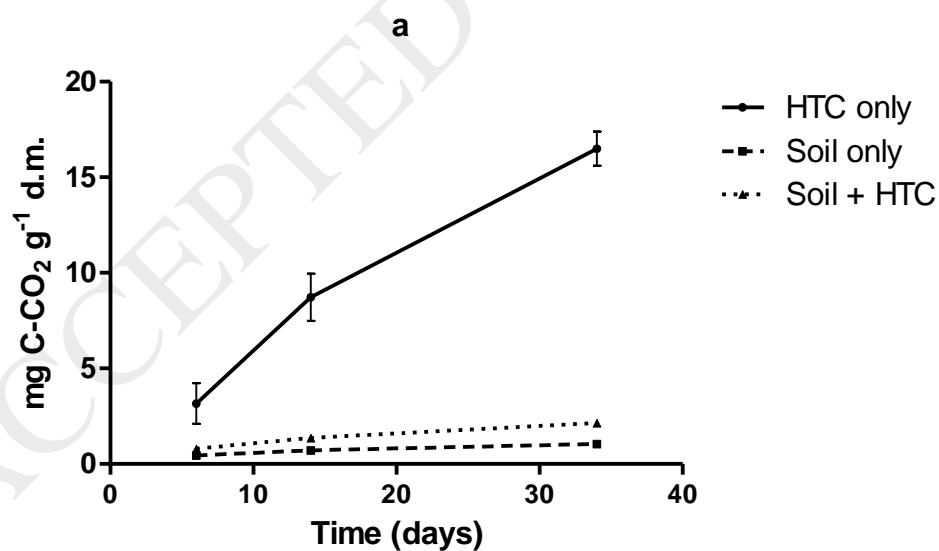


Figure 1



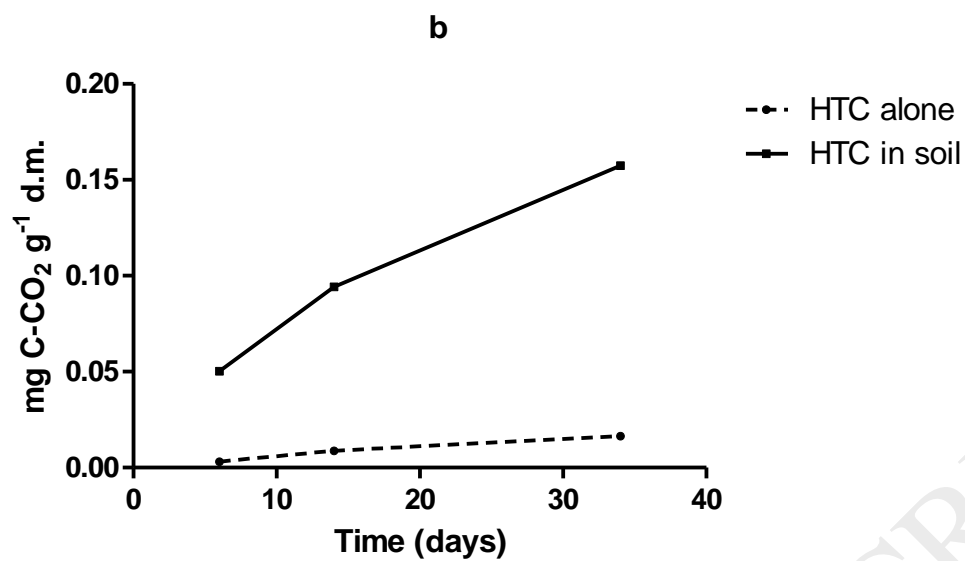


Figure 2a and 2b

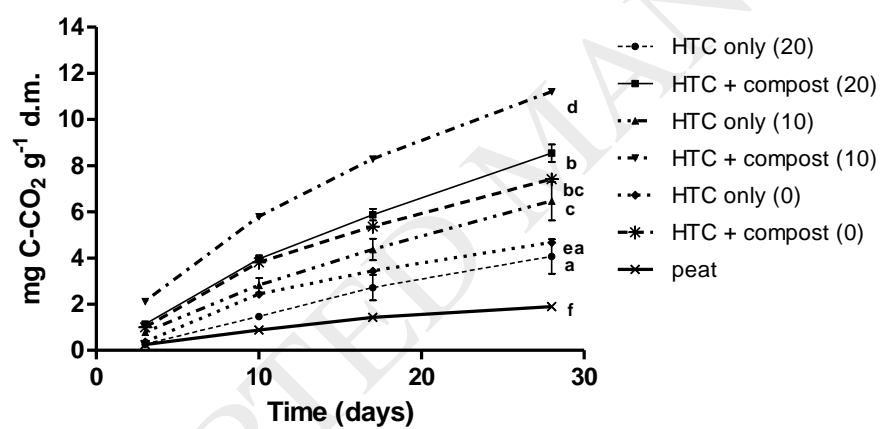


Figure 3

Table 1: Chemical characterization of materials.

Materials	pH	EC	NH ₄ -N mg/L	NO ₃ -N mg/L	P mg/L	K mg/L	Mg mg/L	SO ₄ mg/L	Na mg/L	Mn mg/L	Fe mg/L	Cu mg/L	B mg/L	Zn mg/L
HTC wheat	5.1	246	5	<1	30	283	24	86	19	0.9	2	0.1	0.1	0.5
Biochar maize	8.84	380	98	<1	5	631	8	4	10	0.7	7	0.1	0.1	3.4
Fertilized Peat	4.5-6.0	100-200	126	126	70	350	108	288	0.8	0.8	2.2	0.3	0.6	1

Table 2: Results of first cress seed germination trial on the comparison between HTC and Biochar and the effect of compost addition. Number of seeds germinated and final dry weight per plant is shown in relation to different percentages of compost addition. Last column shows results of statistical comparison. Rows with the same letter indicate non-significant differences between treatments

Treatments	No of seeds germinated						d.w. per plant (mg)	MLV(%)		
Days after seeding										
	2	3	4	7	9	10				
Biochar + 25% compost (v/v)		1	7	8	8	9		0,71	0,37	a
Biochar + 10% compost (v/v)				1	1	1		0,40	0,02	b
Biochar only				1	1	1		0,40	0,02	b
HTC + 10% compost (v/v)	1	14	15	16	16	16		1,56	1,43	c
HTC only		5	12	15	15	15		1,19	1,02	a

Peat		3	7	8	9	10		1,74	1,00	a
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Table 3: Results of second cress seed germination trial on the effect of HTC pre-conditioning. Number of seeds germinated, final dry weight per plant and Munoo-Liisa index is shown in relation to combinations of days of watering prior to seeding and compost addition (20% v/v). Last column shows results of statistical comparison. Rows with the same letter indicate non-significant differences between treatments

Treatments	No of seeds germinated							d.w. per plant (mg)	MLV(%)	
Days after seeding										
	2	3	4	7	9	11	15			
HTC only (20 day pre-conditioning)	2	12	12	12	12	12	12	1,17	0,31	ac
HTC only (10 day pre-conditioning)	2	14	16	16	16	16	16	1,33	0,48	a
HTC only (no pre-conditioning)			2	4	8	10	12	0,90	0,16	b
HTC + compost (20 day pre-conditioning)	2	12	14	14	14	14	14	1,64	0,52	ac
HTC + compost (10 day pre-conditioning)	2	12	12	12	12	12	12	1,58	0,43	acd
HTC + compost (no pre-conditioning)		10	14	14	14	14	14	1,47	0,46	c
peat	14	16	16	16	16	16	16	2,79	1,00	d

Table 4: Results of third cress seed germination trial on the effect of chloroform fumigation. Number of seeds germinated is shown in relation to whether the HTC had been fumigated for 24 h (time 0) or for 10 days prior to seeding. Last column shows results of statistical comparison between treatments at time 0 or 10 days. Rows with the same letter indicate non-significant differences

Treatments		No of seeds germinated					
<i>Days after seeding</i>							
	5	6	7	11	15		
HTC fumigated - time 0	3	7	10	11	11		a
HTC non fumigated - time 0	2	5	7	13	13		a
<i>Days after seeding</i>							
	3	7	8	9			
HTC fumigated - 10 days	0	8	9	10			a
HTC non fumigated - 10 days	1	13	15	15			b